Structural studies on a secretion chaperone from Shigella flexneri and crystallographic explorations with a thermostable aldolase

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Scope of the thesis

Structural characterisation of proteins can lead to a deeper understanding of their functional properties. This thesis contains such structural studies, applied to proteins from three distinct biological systems.

The first part attempts to expand the understanding of the role of secretion chaperones in type III secretion systems (T3S) on a structural level. A type III secretion system is a bacterial protein secretion system, which is used by several bacterial pathogens to inject virulence proteins directly into eukaryotic cells. Prior to injection, several virulence proteins are bound by specific chaperones, whose functional and structural properties are not well-defined. A secretion chaperone from the T3S system of the human pathogen *Shigella flexneri* is structurally characterised. Spa15 has some unusual properties and structural characterisation of this T3S chaperone may allow a better definition of the common principles of chaperoning in this secretion system.

Chapter 1 introduces the *Shigella* type III secretion system and describes how this secretion system is used by *Shigella* to invade human cells and cause dysentery. An overview of chaperones that are encountered in T3S systems will be given, and Spa15 will be introduced. Finally, the contribution of recent structural information, including the investigations described in this thesis, to our understanding of the function of these chaperones is discussed.

Chapter 2 describes the structure of Spa15, determined by X-ray crystallography and small-angle X-ray scattering. Furthermore, the structure is compared with other T3S chaperone structures, which allows identification of some unique structural features of Spa15.

In **Chapter 3** the binding properties of Spa15 are further explored by studying the binding of Spa15 with two of its effectors IpaA and IpgB1. Deletion and limited proteolysis experiments are used to map the Spa15-binding region in IpaA and IpgB1. Spa15:effector complexes are studied by size-exclusion chromatography and fluorescence measurements. These results are used to compare Spa15 binding with other type III secretion chaperones.

The second part commences with **Chapter 4,** which is concerned with *Sulfolobus* 2-keto-3-deoxygluconate (KDG) aldolases. Aldolases are enzymes capable of catalysing the formation of carbon-carbon bonds and thermostable aldolases may be interesting for applications in chemical synthesis. The crystal structure of *Sulfolobus acidocaldarius* KDG aldolase is described and biochemical and structural properties of *Sulfolobus* KDG aldolases are compared and discussed. This allows a detailed description of the structural requirements for its substrates.

During the crystal structure determination process of the KDG aldolase, small red crystals were unexpectedly encountered during some of its crystallisation experiments. **Chapter 5** describes the approach that was used to determine the identity of the protein that had serendipitously formed these crystals. This has eventually led to the determination of a novel crystal form of *Escherichia coli* bacterioferritin. As this represents the first structure of this protein in the form in which it was isolated from the organism, the metal content of this metalloprotein, analysed with X-ray fluorescence measurements, is also described.